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RESEARCH CONCERNING THE BEHAVIOR OF THE *PRUNUS SERRULATA "KANZAN"* VARIETY IN THE PROCESS OF IN VITRO REPRODUCTION

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Abstract: The research that was effectuated has had for a purpose the study of the *Prunus serrulata"Kanzan"* variety behavior during the phases of initiation and in vitro reproduction. In view of the initiation of the culture there have been drawn explants, in the vegetative repose phase. The nutritive media used have had a different content of substances in relation to the in vitro culture phase. For the initiation of cultures the influence of benzylaminopurine was tested, in concentrations of 0,5; 1; 1,5 mg/l. During the microreproduction phase on a constant level of indolebutyric acid and gibberellic acid (0,1 mg/l), was tested the influence of benzylaminopurine (1; 1,4; 2 mg/l). In the room designated for growth were assured controlled temperature conditions (22 - 24^{o}C), photoperiod (12 - 16 hours) and luminosity of 3500 lucs.
The observations and registered data emphasized the influence of BAP over the growth and microreproduction of explants. During the initiation phase of cultures were obtained 95% explants grown with normal aspect. The rate of reproduction increased from 10,6 to 20,3 micro young shoots/explants depending on the increase of cytochinine concentration from 1 to 2 mg/l. The reduction of the photoperiod to 12 hours determined the decrease of the percentage of plants growth and of the rate of reproduction.

INTRODUCTION

Prunus serrulata "Kanzan" is one of the most elegant and appreciated ornamental cherries, the decorative element of the plant being represented by the flowers, which are double, abundant, big, of a deep pink, long petiolated, swinging, and their blossoming is very profuse.

Figure 1
Prunus serrulata "Kanzan"

The use of conventional methods, to multiply this dendrologic variety has a very weak efficiency of conveyance.

At the University of Pitesti, within the laboratory of Vegetal Biotehnologies we undertook studies for the purpose of establishing the in vitro reproduction biotechnology of the *Prunus serrulata* species.

MATERIALS AND METHODS

The biological material used for the initiations of the in vitro cultures was represented by explants composed of meristem and 2 – 3 foliar primordial, drew in the vegetative repose phase.

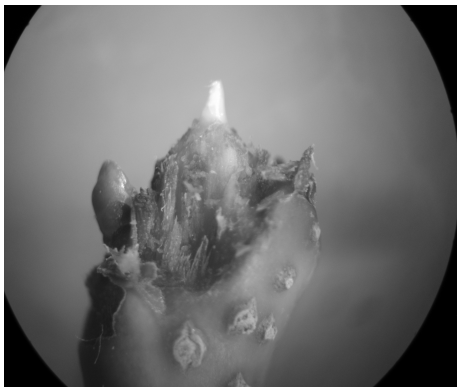


Figure 1, 2

In vitro initiation of *Prunus serrulata "Kanzan"*

The disinfection of the biological material was achieved through:

- washing with tap water adding 2 – 3 drops of Domestos;
- rinsing with tap water;
- keeping in ethylic alcohol 94 % for 10 minutes;
- keeping in calcium hypochlorite 6% for 20 minutes;
- rinsing in distilled water steriliyed by autoclavation.

The drawing of the explants is achieved in aseptic conditions at the hood with laminar air flow under the binocular – eye glass. The nutritive medium used for the iniation of cultures and multiplication of explants have been complex, containing mineral salts grouped as micro and macro elements, vitamins, cytokinines, gigerelines, a source of carbon, and for solidification agar-agar (table 1).

Before the distribution of the nutritive medium in the culture recipients the pH of the medium in the was verified and adjusted to the value of 5,6 – 5,8.

Table 1

Composition of media used for initiation culture and micro propagation

Components (mg/l)	Initiation culture			Micro propagation		
	V_1	V_2	V_3	V_1	V_2	V_3
NH_4NO_3	400	400	400	1650	1650	1650
KNO_3	1800	1800	1800	1900	1900	1900
$MgSO_4$	360	360	360	370	370	370
KH_2PO_4	270	270	270	170	170	170
$Ca(NO_3)_2$	1200	1200	1200	-	-	-
$CaCl_2$	-	-	-	440	440	440
$MnSO_4$	0,75	0,75	0,75	22,3	22,3	22,3
$ZnSO_4$	8,6	8,6	8,6	8,6	8,6	8,6
H_3BO_3	12,0	12,0	12,0	6,2	6,2	6,2
$CuSO_4$	0,025	0,025	0,025	0,025	0,025	0,025
Na_2MoO_4	0,25	0,25	0,25	0,25	0,25	0,25
$CoCl_2$	0,025	0,025	0,025	0,025	0,025	0,025
KI	0,08	0,08	0,08	0,83	0,83	0,83
Inositol	100	100	100	54,048	54,048	54,048
Nicotinic acid	-	-	-	2,462	2,462	2,462
Pyridoxine hydrochloride	-	-	-	0,616	0,616	0,616
Thiamine hydrochloride	0,4	0,4	0,4	0,674	0,674	0,674
Biotin	-	-	-	0,048	0,048	0,048
Panthotenic acid calcium	-	-	-	0,476	0,476	0,476
Riboflavin	-	-	-	0,376	0,376	0,376
Ascorbic acid	-	-	-	0,176	0,176	0,176
Choline chloride	-	-	-	0,104	0,104	0,104
Cysteine	-	-	-	7,269	7,269	7,269
Glycine	-	-	-	0,375	0,375	0,375
Gibberellic acid	-	-	-	0,1	0,1	0,1
3 indolebutyric acid	-	-	-	0,1	0,1	0,1
Benzilaminopurin (BAP)	0,5	1	1,5	1	1,4	2,0
NaFeEDTA	32,0	32,0	32,0	32,0	32,0	32,0
Sucrose (g/l)	40,0	40,0	40,0	40,0	40,0	40,0
Agar (g/l)	7,0	7,0	7,0	7,0	7,0	7,0

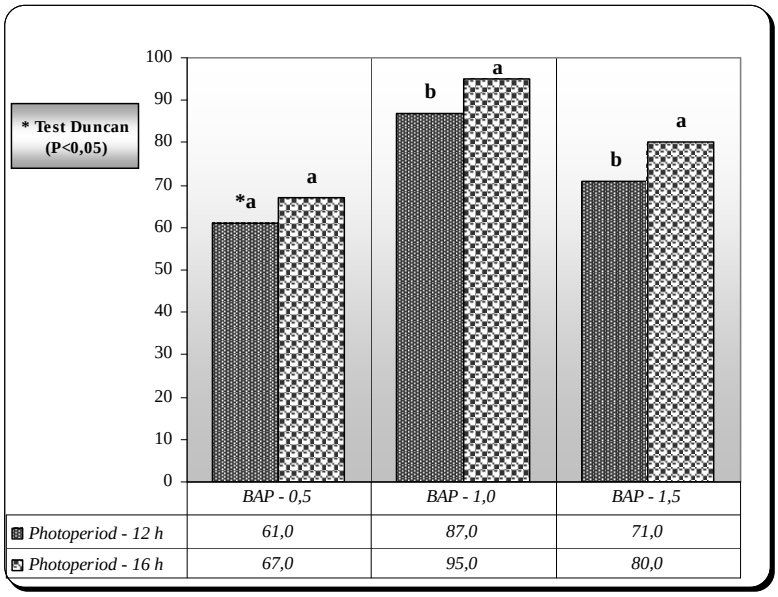
The steriliying of the nutritive sublayer was achieved through autoclavation at one atmosphere (121°C) for 20 minutes. The culture recipients, instruments, cassolettes have been steriliyed in the air oven (120°C for 2 hours). The growing and multiplication of explants was achieved in controlled conditions of temperature (22 - 24°C), photoperiod (12 – 16 hours), luminous intensity 3500 lucs.

The recorded data have been expressed in percentages of growth of the explants in the initiation phase of the cultures and ratio of multiplication (micro young shoots/explants) in the multiplication phase.

RESULTS AND DISCUSSIONS

In the iniation phase and microreproduction the growth and reproduction was influenced by the composition of the nutritive medium and photoperiod thus.

The notes and recorded data emphasized on a constant level of the photoperiod of 16 hours that the increase of the concentration of BAP from 0,5 to 1 mg/l has caused the growth of explants in a percentage of 60 to 95%.



	BAP - 0,5	BAP - 1,0	BAP - 1,5
Photoperiod - 12 h	61,0	87,0	71,0
Photoperiod - 16 h	67,0	95,0	80,0

Figure 3
Growing of explants in function of nutritive medium composition and photoperiod

The increase of the concentration BAP to 1,5 mg/l has influenced negatively the growth of explants whose value reached 80 %, the explants presenting the phenomenon of callus formation and vitrification.

The decrease of the photoperiod from 16 to 12 hours led to the achievement of some smaller percentages of grown explants, which depending on the BAP concentration, have been included between 61 at the concentration of 0,5 and 87 at the concentration of 1mg/l.

In this case the BAP concentration of 1,5 mg/l has caused the phenomenon of callus formation and vitification and as a consequence the decrease of the growth percentages of the explants to the value of 71.

From the obtained results for the microreproduction phase it was found that ratio of multiplication has increased together with the increase of the BAP concentration from 8,0 to 20, 3 micro young shoots/explants regardless of the photoperiod.

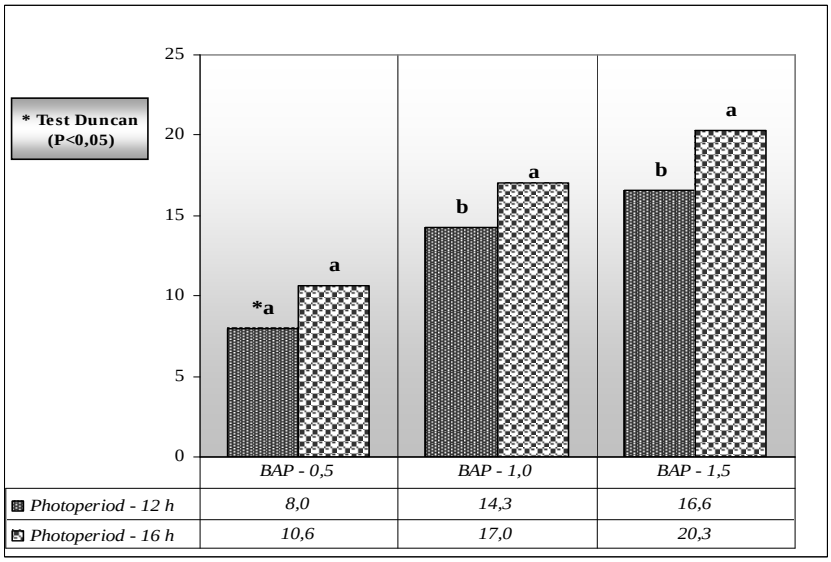


Figure 4
Rate of multiplication in function of nutritive medium composition and photoperiod

Although, for the third version (2,0 mg/l BAP) a number of 20,3 micro young shoots/explants was obtained, these manifested the phenomenon of callus formation and vitrification comparing to V_2 (1,4 mg/l BAP) where RI was of 17 micro young shoots/explants but the plants have had a normal aspect.

Analyzing the influence of the second variable factor, the photoperiod, it is found there are positive results through the increase from 12 to 16 hours photoperiod, the number of micro young shoots/explants has increased in V_2 from 14,3 to 17.

Fig.5 – Aspect of multiplication stage of *Prunus serrulata "Kanzan"*

Fig.6 – Rate of multiplication at the *Prunus Serrulata "Kanzan"*

CONCLUSIONS

The results regarding the initiation and multiplication phases of the *Prunus serrulata "Kanzan"* variety have led to the following conclusions:

- in the initiation phase the best results were obtained in the presence of BAP in the concentration of 1 mg/l;

- for microreproduction, the use of a basic were medium Linsmaier – Skoog (1965) with an addition of fitohormones (0,1 mg/l GA and IBA; 1,4 mg/l BAP) has led to the highest rates of multiplication;
- as far as the influence of the photoperiod in the two phases is concerned, the best results were obtained in an illumination of 16 hours.

REFERENCES

1. Isac, Maria, 1983, Comportarea unor soiuri de prun în faza de multiplicare in vitro, Piteşti – Lucrările celui de al II – lea Simpozion Național de culturi de țesuturi vegetale ”In Vitro”, Vol. II, 212 – 218.
2. Linsmaier, E. M., F. Skoog, 1965, Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant, p. 100 -127.
3. Posedaru, Elena Alina, 2005, Plante ornamentale recomandate pentru amenajarea spațiilor verzi din România, Ed. Universității din Piteşti, p. 137-140.
4. Pinker, L., 1997, Mikrovermehrung von *Prunus serrulata* ”Kanzan”, Deutsche Baumschule, 8: 426-427.
5. Ones, O. P., M. E. Hopgood, 1979, The successful propagation in vitro of two rootstocks of *Prunus*: the plum rootstock Pixy (*P. insititia*) and the cherry rootstock F12/1 (*P. avium*)" *Journal of Horticultural Science* 54 (1), 63-66.
6. Posedaru, Alina, Mădălina Militaru, 2001, Studii cu privire la asigurarea decorului cu specii şi varietăți dendrologice valoroase în parcul dendrologic Piteşti-Mărăcineni, Analele Universității din Craiova, Vol. VI (XLII), I.S.S.N. 1435-1275.